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FORM-PTO-1390 (Rev. 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 012627-020

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/744186

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE99/02280	21 July 1999	21 July 1998

TITLE OF INVENTION: AGENTS FOR THE IMMUNOTHERAPY OF TUMORAL DISEASES

APPLICANT(S) FOR DO/EO/US: Aida KERKMANN-TUCEK

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

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U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/744186	PCT/DE99/02280	012627-020

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	13 -20 =	0	X$18.00 (966)	$	--
Independent Claims	1 -3 =	0	X$80.00 (964)	$	--
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$	270.00
TOTAL OF ABOVE CALCULATIONS =				$	1130.00
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	565.00
SUBTOTAL =				$	565.00
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	565.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$	
TOTAL FEES ENCLOSED =				$	565.00
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 565.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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K 2928 US

Agents for the Immunotherapy of Tumoral Diseases

The present invention relates to agents suitable for the immunotherapy of tumoral diseases. These agents are tumor cells, a tumor cell library containing them and vaccines containing said tumor cells. The invention also relates to a method for producing the tumor cells and to the use of these cells and the vaccines and the tumor cell library.

The most varying methods are used or treating tumoral diseases. Primary tumors are often removed by means of surgery, and the patients are subjected to a follow-up in the form of a chemotherapy and/or a radiation therapy. The follow-up shall serve for destroying residual tumor cells. Immunotherapy methods are also employed in which tumor cells obtained from primary tumors are manipulated and returned to the patients. This shall serve for sensitizing the immune system to the tumor cells so as to prevent subsequent formation of metastases. However, the immunotherapy methods do not yet show the desired results. The sensitization of the immune system often fails to suffice, so that tumor cells remain unidentified and metastases may develop. Immunotherapy methods also require the use of patient-inherent tumor cells for the treatment. This is very costly and time-consuming. Immunotherapy often cannot be carried out at all because it is not possible to collect sufficient tumor cells from the individual patient.

Therefore, it is the object of the present invention to provide an agent by means of which immunotherapy of tumoral diseases can be carried out, the above drawbacks being avoided.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on Applicant's insights regarding the correlation between the expression of MHC ("major histocompatibility complex") I, II genes in tumor cells and the immunogenicity thereof. MHC I genes are also referred to as HLA-A, HLA-B and HLA-C genes. Furthermore, MHC II genes are also referred to as HLA-Dr, HLA-DQ, and HLA-DP genes. Applicant recognized that the expression of MHC I and/or MHC II genes is disturbed in tumor cells. In particular, he found that many tumor cells do not express MHC II genes. He also found that because of the disturbed expression of the MHC I and/or MHC II genes and the accompanying lack of corresponding gene products on the surface of tumor cells, the tumor antigens present on this surface are not recognized as foreign antigens by the immune system and therefore the tumor cells are not destroyed. On the other hand, Applicant discovered that tumor cells which have a combination of MHC I and MHC II genes, occurring in humans, and also express it, show a high degree of immunogenicity. Such combinations are particularly those indicated in Table I. He also discovered that the immunogenicity of such tumor cells can even be increased if they also express co-stimulatory molecules and/or cytokines.

According to the invention Applicant's insights are utilized to provide tumor cells with a combination of MHC I and MHC II genes, occurring in humans, which genes are expressed.

The expression "tumor cells" comprises tumor cells of any tumor occurring in man. Examples of tumors comprise mammary carcinomas, anogenital carcinomas, lung carcinomas, colon carcinomas, brain tumors, gastric carcinomas, bladder carcinomas, liver cell carcinomas and melanoma.

The tumor cells may be freshly isolated or be present in culture. They may also be present as such or in a cell aggregate, e.g. (primary) tumor or metastasis.

The expression "combination of MHC I and MHC II genes" comprises any combination of MHC I and MHC II genes which may occur in humans. In particular, the combination is selected from the combinations indicated in Table I.

The term "expressed genes" refers to the fact that the combination of MHC I and MHC II genes is expressed. This can be achieved by common methods. It is favorable to initially subject the tumor cells and optionally other cells, e.g. lymphocytes, from the same patient to tissue typing so as to determine which of the MHC I and/or MHC II genes show a disturbed expression. Tissues may be typed e.g. by serological methods, as known from the "11th International Histocompatibility Workshop". The disturbed expression of the MHC I and/or MHC II genes may then be compensated by transfection of corresponding exogenous genes, optionally present on expression vectors, in the tumor cells. Examples of expression vectors comprise pUHD10-I, pRcRSV, pBSK, RSV.5 hygro, pBJ and B45-neo, and examples of transfection methods are calcium phosphate co-precipitation, electroporation, lipofection, DOTAP liposomes and retroviral transfection. The expression of transfected MHC I and/or MHC II genes may be stable or transient, stable ones being preferred. The expression may be detected by common methods, e.g. serological methods, see above.

In a preferred embodiment, the above tumor cells also have one or several genes which code for co-stimulatory molecules and/or cytokines, which are expressed. Examples of co-stimulatory molecules are B7, such as B7-1 or B7-2, and CD44. Examples of cytokines comprise interleukins, such as IL-2, GM-CSF, TNF-α and interferon-γ. The presence of the addressed genes in the tumor cells and the expression of the genes may be achieved as usual. Reference is made to the above statements.

A further subject matter of the present invention relates to a method of producing the above tumor cells. Such a method comprises the steps of:

(a) tissue typing of tumor cells,
(b) transfection of the tumor cells using MHC I and/or MHC II genes so as to obtain a combination of these genes, occurring in humans, and
(c) selection on the tumor cells which express the MHC I and MHC II genes.

The expression "tissue typing of tumor cells" comprises any method serving for determining the expression of MHC I and MHC II genes. Reference is made to the above statements. It may be favorable to subject cells, e.g. lymphocytes, of the same patient from which the tumor cells originate to tissue typing. This facilitates a detection of the combination of MHC I and MHC II genes suitable for this patient.

The expression "transfection of tumor cells" comprises any method by which MHC I and/or MHC II genes can be transfected in tumor cells. Reference is made to the above statements.

The expression "selection on tumor cells" comprises any methods serving a selection on tumor cells which express MHC I and MHC II genes. Reference is made to the above remarks.

In a preferred embodiment, the tumor cells are also transfected with one or several genes coding for co-stimulatory molecules and/or cytokines and selected for the expression of these genes. Reference is made to the above remarks.

A further subject matter of the present invention relates to a tumor cell library comprising the above tumor cells. The tumor cells preferably originate from any human tumor and comprise any combination of MHC I and MHC II genes occurring in man. It is particularly preferred that the tumor cells

comprise the combinations of MHC I and MHC II genes, indicated in Table I.

A further subject matter of the present invention relates to a vaccine comprising the above tumor cells and conventional auxiliary agents, e.g. buffers, carriers and diluents. The vaccine preferably contains tumor cells of different tumors with equal combination of MHC I and MHC II genes each.

The present invention provides tumor cells which express a combination of MHC I and MHC II genes, the combination occurring in a human being. In particular, such a combination occurs in many humans. Thus, the tumor cells according to the invention represent a product which cannot be administered merely to a certain human being but can be given to many humans. In addition, the tumors originate from any human tumor. In so far, the present invention is not limited to the treatment of a certain tumor but can be used for a wide range of tumors.

By means of the present invention it is possible to offer immunotherapy to patients suffering from a tumoral disease. The major advantage of this therapy is that it can be carried out rapidly. Having determined the kind of tumor and its typing or other cells originating from the same patient, suitable tumor cells according to the invention can be selected, e.g. from the tumor cell library, and be administered to the patient. Before administering the tumor cells, it is favorable to prevent them from replicating by certain measures, such as irradiation.

Furthermore, the present invention enables prophylactic steps to be taken against all kinds of tumors. For this purpose, it is merely necessary to type the tissue of cells from the human to be treated and then give the latter a suitable vaccine according to the invention.

Thus, the present invention is a break-through for the immunotherapy of tumoral diseases.

The invention is explained by the below example.

Example: Preparation of tumor cells according to the invention

(A) Establishing tumor cells of a melanoma patient

The tumor of a melanoma patient is removed, comminuted and placed in several cell culture bottles containing DMEM medium. After 48 hours of culturing (37°C, 5 % CO_2), the medium is exchanged. Five cell culture bottles are selected after 1-2 weeks, which are treated independently.

(B) Tissue typing of lymphocytes and tumor cells of a melanoma patient

1. Making lymphocytes and tumor cells ready for tissue typing.

Blood is withdrawn from melanoma patients from (A). This blood is supplied with an anti-coagulant and diluted with the same volume of HBSS solution. The blood is filled into small tubes which already contain ficoll. The tubes are centrifuged at 800 g for 20 min. The resulting interphase is removed, resuspended in HBSS solution, and centrifuged at 500 g for 5 min. The resulting lymphocytes are counted and standardized for tissue typing as a solution having a concentration of 1-2 x 10^6/ml.

The established tumor cells from (A) are standardized in equal concentration.

2. Serologic determination of HLA molecules on lymphocytes and tumor cells

Polystyrene plates are used which are coated with antisera against HLA-A, HLA-C, HLA-B, HLA-DR, HLA-DQ and/or HLA-DP. 1 µl of the lymphocyte solution or tumor cell solution from

(B) 1. is added to each of these plates. The plates are incubated at 22°C for 30 min. before 5 µl fresh complement are added each. Then, the plates are incubated at 22°C for 60 min. before 1 µl of an acridine orange/ethidium bromide cocktail and 1 µl of quencher solution are added each. The plates are allowed to stand at room temperature for 4 hours. Positive samples are shown by the development of fluorescent staining.

It shows that the lymphocytes comprise the following HLA molecules:
A*01; Cw*07; B*08; DRB1*03; DQA1*02; DQB1*02; DPA1*02; DPB1*02.

The tumor cells, however, only have the following HLA molecules:
A*01; Cw*07; B*08.

3. Determining the HLA genes of lymphocytes

RNA is isolated from the lymphocytes from (B) 1. and subjected to reverse transcription. The resulting cDNA is subjected to a PCR method which uses primer groups that are selected in accordance with the HLA molecules from (B) 2. In particular, the following primers are used:

A*01:	forward:	CGA CGC CGC GAG CCA GAA
	reverse:	AGC CCG TCC ACG CAC CG
Cw*07:	forward:	GGA CCG GGA GAC ACA GAA C
	reverse:	CGC ACG GGC CGC CTC CA
B*08:	forward:	GAC CGG AAC ACA CAG ATC TT
	reverse:	CCG CGC GCT CCA GCG TG
DRB1*03:	forward:	GAC GGA GCG GGT GCG GTA
	reverse:	CTG CAC TGT GAA GCT CTC CA
DQA1*02:	forward:	CGA GTT TTA CGG TCC CTC TGG C

reverse: CTC ATT GGT AGC AGC GGT AGA GTT GG

DQB1*02: forward: GTG CGT CTT GTG AGC AGA AG
reverse: CGT GCG GAG CTC CAA CTG

DPA1*02: forward: CCC GCT CTG GTT TGA TTT AT
reverse: CAC TTC GCA TCT ATG CGA

DPB1*02: forward: AGG ACA GAA CTC GGT ACT AGG A
reverse: TGA ATC CCC AAC CCA AAG TCC CC

The PCR conditions are as follows: 24 cycles: 1 min, 94°C; 45 sec, 65°C; 2 min, 72°C. Final cycle: 1 min, 94°C; 45 sec, 65°C; 10 min, 72°C.

The amplified DNA is cleaved by the restriction enzymes SalI and HindIII and inserted in the correspondingly cleaved vector M13 mp18 or M13 mp19. Recombinant DNA molecules are used for the transformation of *E. coli* JM109. Resulting clones are subjected to a screening method by means of hybridization with the amplified DNA. Positive clones are subjected to sequencing.

It shows that the HLA molecules from (B) 2. are encoded by the following HLA genes:

A*0101; Cw*0701; B*0801; DRB1*0301; DQA1*0201; DQB1*0201; DPA1*0201; DPB1*0201.

4. Isolation of HLA-D genes from lymphocytes and transfection thereof in tumor cells

Blood is withdrawn from the melanoma patient from (A). This blood is supplied with an anti-coagulant and diluted with eight times an excess of RCL buffer. The blood is centrifuged in a microcentrifuge for 30 sec. The pellet is taken up with RCL buffer and centrifuged. Having repeated this step several times, the pellet is dissolved in NLB buffer and incubated with proteinase K at 63-65°C for 1 hour

and at 95°C for 10 min. The solution is centrifuged in a microcentrifuge for 60 sec and the pellet is discarded. The supernatant contains the DNA from the lymphocytes.

This DNA is subjected to a PCR method which employs the primers used in (B) 2. for the HLA-D genes, DRB1*03, DQA1*02, DQB1*02, DPA1*02 and/or DPB1*02.

The PCR conditions are as follows: 30 cycles; 30 sec, 98°C; 60 sec, 55°C; 105 sec, 72°C. Final cycle: 7 min., 72°C.

Samples of the amplified DNA are separated on a 1 % agarose gel by means of electrophoresis. This yields fragments of 216 bp for DRB1*03, of 219 bp for DQA1*02/DQB1*02, and of 245 bp for DPA*02/DPB1*02.

The amplified DNA is cleaved by the restriction enzymes SalI and HindIII and inserted in the correspondingly cleaved expression vector B45-neo. Recombinant DNA molecules are used for the transformation of *E. coli* JM109 or DH5F'α. Resulting clones are subjected to a screening method by means of hybridization using the amplified DNA. Positive clones are confirmed by sequencing.

These clones are used for the transfection of the tumor cells from (A). The tumor cells are trypsinated, and electroporation is carried out with 400 V and 490 µ FD. The tumor cells are selected with G418 (400 - 100 µg/ml) for 4 weeks before they are subjected to "fluorescence activating cell sorting" (FACS). Tumor cells are obtained which express the following HLA molecules:

A*0101; Cw*0701; B*0801; DRB1*0301; DQA1*0201; DQB1*0201; DPA1*0201; DPB1*0201.

5. Transfection of tumor cells with genes coding for co-stimulatory molecules and/or cytokines

cDNAs which code for CD44, IFN-γ and/or GM-CSF are obtained from Invitrogen company. The cDNAs are inserted in the expression vectors RSV.5 hygro (blunt/BamHI), pUHD10-1 (XmnI) and/or pBSK (BamHI). Recombinant DNA molecules are used for transforming *E. coli* JM109 or DH5'α. Resulting clones are subjected to a screening method by means of hybridization with the cDNAs. Positive clones are confirmed by sequencing.

These clones are used for transfecting the tumor cells obtained in (B) 4. The transfection is carried out with DOTAP liposomes according to the instructions from the manufacturer Boehringer Mannheim. The transfected tumor cells are screened by FACS and/or RT-PCR. Tumor cells are obtained which express the following HLA molecules and co-stimulatory molecules as well as cytokines:

A*0101; Cw*0701; B*0801; DRB1*0301; DQA1*0201; DQB1*0201; DPA1*0201; DPB1*0201; IFN-γ; DC44; GM-CSF.

Table I

Frequent HLA Combinations

People	HLA-A	HLA-C	HLA-B	HLA-DP	HLA-DQ	HLA-DP	Frequency
Cornish	A*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0101	8.4%
German	A*-0101	Cw*-0701	B*-5801	DR*-0301	DQ*-0201	DP*-0401	4.8%
German	A*-0101	Cw*-0701	B*-0801	DR*-1501	DQ*-0101	DP*-0401	2.5%
USA	A*-1001	Cw*-0701	B*-0801	CR*-0301	DQ*-0201	DR*-0901	4.3%
Canadian	A*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0101	5.1%
Australian	A*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0101	7.6%
Japanese	A*-2401	CBL	B*-5201	DR*-1501	DQ*-0101	DP*-0901	8.2%
Japanese	A*-3301	CBL	B*-4401	DR*-1302	DQ*-0101	DP*-0401	4.9%
Indian	A*-2401	CBL	B*-6101	DR*-1501	DQ*-0101	DP*-0402	4.1%
Thais	A*-0201	Cw*-1101	B*-4601	DR*-0901	DQ*-0301	DP*-0401	4.5%
Taiwan	A*-2401	Cw*-0701	B*-3901	DR*-1201	DQ*-0701	DR*-0301	10.4%
Inuit	A*-2401	CBL	B*-4801	DR*-0401	DQ*-0701	DP*-0201	9.4%
Singapore	A*-0201	Cw*-1101	B*-0801	DR*-0301	DQ*-0301	DP*-0401	7.2%
Maori	A*-0801	Cw*-0101	B*-5501	DR*-1201	DQ*-0701	DP*-0101	8.1%
Bushman	A*-3001	CW*-0401	B*-5801	DR*-1301	DQ*-0101	DP*-0401	8.2%
North Am.-Negroid	A*-3601	CW*-0401	B*-5301	DR*-1101	DQ*-0101	DP*-0101	1.1%

Bas-que	A*-2901	CBL	B*-4401	DR*-0701	DQ*-0201	DP*-0201	5.4%
Java-nese	ABL	CBL	B*-6201	DR*-1201	DQ*-0701	DP*-0401	8.2%
Mon-goli-an	A*-3001	Cw*-0601	B*-1301	DR*-0701	DQ*-0201	DP*-0201	4.0%
Ura-lic	A*-1101	CW*-0401	B*-3501	DR*-0301	DQ*-0201	DP*-0101	3.1%

Claims

1. Tumor cells comprising a combination of MHC I and MHC II genes, occurring in humans, which genes are expressed.

2. The tumor cells according to claim 1, wherein one or several genes are also expressed for co-stimulatory molecules.

3. The tumor cells according to claim 2, wherein the co-stimulatory molecules comprise B7 and CD44.

4. The tumor cells according to any of claims 1 to 3, wherein one or several genes are also expressed for cytokines.

5. The tumor cells according to claim 4, wherein the cytokines are interleukins, GM-CSG, TNF-α and interferon-γ.

6. The tumor cells according to any of claims 1 to 5, wherein the combination of MHC I and MHC II genes is selected from the group consisting of:

Peo-Ple	HLA-B	HLA-C	HLA-B	HLA-DR	HLA-DQ	HLA-DR	Fre-quen-cy
Cor-nish (Kel-ten)	DP*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0101	8.4%
Ger-man	A*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0401	4.8%
Ger-man	A*-0101	Cw*-0701	B*-0701	DR*-1501	DQ*-0101	DP*-0401	2.5%
USA	A*-1001	Cw*-0701	B*-0801	CR*-0301	DQ*-0201	DP*-0401	4.3%
Cana-dian	A*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0101	5.1%
Au-stra-	A*-0101	Cw*-0701	B*-0801	DR*-0301	DQ*-0201	DP*-0101	7.6%

lian							
Japanese	A*-2401	CBL	B*-5201	DR*-1501	DQ*-0101	DP*-0901	8.2%
Japanese	A*-3301	CBL	B*-4401	DR*-1302	DQ*-0101	DP*-0401	4.9%
Indian	A*-2401	CBL	B*-6101	DR*-1501	DQ*-0101	DP*-0402	4.1%
Thais	A*-0201	Cw*-1101	B*-4601	DR*-0901	DQ*-0301	DP*-0401	4.5%
Taiwan	A*-2401	Cw*-0701	B*-3901	DR*-1201	DQ*-0701	DP*-1301	10.4%
Inuit	A*-2401	CBL	B*-4801	DR*-0401	DQ*-0701	DP*-0201	9.4%
Singapore	A*-0201	Cw*-1101	B*-4601	DR*-0901	DQ*-0301	DP*-0401	7.2%
Maori	A*-0201	Cw*-0101	B*-5501	DR*-1201	DQ*-0701	DP*-0101	8.1%
Bushman	A*-3001	CW*-0401	B*-5801	DR*-1301	DQ*-0101	DP*-0401	8.2%
North Am.-Negroid	A*-3601	CW*-0401	B*-5301	DR*-1101	DQ*-0101	DP*-0101	1.1%
Basque	A*-2901	CBL	B*-4401	DR*-0701	DQ*-0201	DP*-0201	5.4%
Javanese	ABL	CBL	B*-6201	DR*-1201	DQ*-0701	DP*-0401	8.2%
Mongolian	A*-3001	Cw*-0601	B*-1301	DR*-0701	DQ*-0201	DP*-0201	4.0%
Uralic	A*-1101	CW*-0401	B*-3501	DR*-0301	DQ*-0201	DP*-0101	3.1%

7. The tumor cells according to any of claims 1 to 6, comprising the following combination of MHC I and MHC II genes:

A*0101; Cw*0701: B*0801; DRB1*0301; DQA1*0201; DQB1*0201; DPA1*0201; DPB1*0201.

8. The tumor cells according to any of claims 1 to 7, comprising the following combination of MHC I/II genes and genes for IFN-γ, CD44 and GM-CSF:

A*0101; Cw*0701; B*0801; DRB1*0301; DQA1*0201; DQB1*0201; DPA1*0201; DPB1*0201; IFN-γ; CD44; GM-CSF.

9. A method for producing the tumor cells according to claim 1, comprising the steps of:

(a) tissue typing of tumor cells,
(b) transfection of the tumor cells with MHC I and/or MHC II genes so as to obtain a combination of these genes, occurring in humans, and
(c) selection for tumor cells which express the MHC I and MHC II genes.

10. The method according to claim 9, wherein the tumor cells are further transfected with one or several genes coding for co-stimulatory molecules and/or cytokines and selected for the expression of these genes.

11. Tumor cell library, comprising the tumor cells according to any of claims 1 to 8.

12. Vaccines, comprising the tumor cells according to any of claims 1 to 8 and conventional auxiliary agents.

13. Use of the tumor cells according to any of claims 1 to 8, of the tumor cell library according to claim 11 or of the vaccines according to claim 12 for the prophylaxis and/or treatment of tumoral diseases.

Abstract of the Disclosure

The present invention relates to agents suitable for the immunotherapy of tumoral diseases. These agents are tumor cells with a combination of MHC I and MHC II genes, occurring in humans, which genes are expressed. The invention further relates to a tumor cell library comprising the above tumor cells and to vaccines containing said tumor cells. The invention also relates to a method for producing the tumor cells and to the use of said cells and of the vaccines and the tumor cell library.

K2928

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 012627-020

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AGENTS FOR THE IMMUNOTHERAPHY OF TUMORAL DISASES

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number _____
on _____
and was amended
on _____ (if applicable).

☒ was filed as PCT international application
Number PCT/DE99/02280
on 21 July 1999
and was amended
on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Germany	198 32 840.0	21 July 1998	X Yes _No
			_Yes _No
			_Yes _No
			_Yes _No
			_Yes _No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ _____
(Application Number) (Filing Date)

_____ _____
(Application Number) (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 012617-020

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Eric H. Weisblatt	30,505	Bruce T. Wieder	33,815
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 012617-020

FULL NAME OF SOLE OR FIRST INVENTOR Aida KERKMANN-TUCEK	SIGNATURE Aida Kerkmann	DATE 02.18.2004
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FULL NAME OF SECOND JOINT INVENTOR, IF ANY	SIGNATURE	DATE
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FULL NAME OF THIRD JOINT INVENTOR, IF ANY	SIGNATURE	DATE
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FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		